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Abundance, Distribution and Diversity of *Aspergillus* associated with cashew nut plantations in Coastal Sand Dunes, Odisha, India

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ABSTRACT:
This paper reports abundance and diversity of Aspergilli in monoculture plantation of *Anacardium occidentale* L. in coastal sandy soils of Odisha, India for a period of two years. A total of 22 species were enumerated of which surface soil of barren sand dune had a share of 16 species while sub surface soil produced 11 species. Surface soil with *Anacardium* plantation contributed 13 species while sub surface soil contributed 14 species. More species were recorded from unproductive coastal sand dunes in spite of its low nutrient status, which may be due to less competition with other fungi. The diversity index varied from 0.764 to 0.917(Shannon) and 0 .0102 to 0.0148 (Simpson).The similarity index showed that surface layers was found to be more akin than the subsurface soils. The evenness index varies from 0.332to 0.452 an indication that species were fairly evenly distributed. The surface soil without plantation has the highest species richness where as sub-surface soil of barren sand dunes shows lowest richness.

Key words: *Anacardium occidentale*, *Aspergillus*, Coastal sand dune, Diversity indices, Fungi

INTRODUCTION

Soil fungal diversity and population density are often a reflection of methods used to recover the fungi; with optimal sampling methods differing from organism to organism [1, 2]. It is very well experimented and concluded that soil fungal diversity and population density are invariably the reflections of the methods used to recover the fungi. Identification of fungi is complicated as the fungal life cycle in natural habitat is quite different from the laboratory [3].Furthermore; fungi are so nutritionally diverse that there is no one medium that can isolate all of them. Though we are exploring the fungi in a faster rate with modern techniques; but till now we have identified only 50% of the Indian species which is expected to be one third of the fungi found in the globe [4, 5]. The patent literature on fungal diversity concluded that different habitat exhibited variation, among different plant systems and also both environment and edaphic factors greatly influence the growth and development of microbes [6-10]. It is disappointing that despite a long period of research many fungi are yet to be discovered. In this context the lack of research in coastal sand dunes is surprising in comparison to forest soils [7, 9, 11-15]. In view of the above fact a study was carried out and summarizes here the role of beach plantation in determining the abundance and diversity of fungal flora in general and genus *Aspergillus* in particular in coastal sand dunes of Odisha with tree plantation of *Annacardium occidentale*.

MATERIALS AND METHODS

The study site was in Ganjam district of Odisha (19º15'N and 84º50'E) having 60km of coastline along the Bay of Bengal at a height of 6-8m above MSL. The climate of the region is monsoonal with coastal characteristics. The air temperatures ranges from 37°C in summer to 13°C in winter with an annual average rainfall of approximately 130cm.Some of the unproductive uplands and coastal sand dunes are extensively covered by *Casuarina equisetifolia* and Cashew (*Anacardium occidentale*) plants. Cashew plantation at the inner belt of study site covers an area of about 1500 hectares extending 4-5 km with a width of 250-450 m, varying at places and a shelter belt cum wind break vegetation of *Casuarina* about 30-40 rows covering 15-20 m in the outer belt along the coast of the sea. Cashew plant has been preferred over many others because of its physiological adaptation to tolerate extreme drought conditions, good growth in nutritionally poor soils, extensive near surface lateral roots and dense canopy due to broad leaf and horizontal growth. Two sites of about one hectare each were selected for the investigation. First one is a big patch of sand dune situated adjacent to *Anacardium* plantation comprising few grasses only and the second along a coastal sandy bed with 6-8 yr old plantation of *Anacardium occidentale* without any undergrowth. The study was conducted for a period of two years. Soil samples from surface and sub-surface (15 cm depth) were collected from two sites in sterilized test tubes by randomly sampling at monthly intervals. The samples were temporarily stored in an ice chest prior to isolation of microbes. The micro fungi were isolated by dilution [16] and pour plate [17] techniques using PDA medium. Fungi were studied after 3-7 days of incubation. Fungi were identified by adopting standard procedures [18-23]. Physico-chemical properties of soils were estimated as per Jackson (1967) [24].

STATISTICAL ANALYSIS

The following indices of diversity were calculated based on species level identification [25].

Shannon –Wiener index $H = -\sum_{i=0}^{s} PilnPi$

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where Pi is the proportion of the individual found in the i[th] species, ln denotes natural logarithm and H is the Shannon –Wiener index .

Simpson's index $D = \sum_{i=0}^{s}(Pi)^2$

Where Pi is the proportion of the individual found in the i[th] species and D Simpson's index

Evenness index (E) = H/lnS

Where H is the Shannon –Wiener index of diversity, S total number of species and ln is the natural logarithm.

Jacquard's index $S_{ab} = S_{AB} / (S_A + S_B - S_{AB})$

Where S_{AB} is the number of species shared by two locations(A and B), S_A the total number of species in location A and S_B the total number of species in location B. S_{ab} is the extent of similarity between the species in location A and B.

Richness index[26] R =S-1/lnN

Where S is the total number of species and N is the sampling number.

RESULTS AND DISCUSSION

The aim of the study was determination of *Aspergillus* distribution and diversity associated with *Anacardium occidantale* in coastal sand dunes of Odisha. Study of plant and fungal diversity is very important to biological conservation, monitoring forest dynamics, forest management and ecorestoration[27-32].Considering that no article was found similar to this study on coastal sand dunes associated with *Anacardium occidantale*, therefore two sites were selected for comparison. Coastal dune environment is always changing, but it is important to realize that change is natural and health part of the ecosystem and helps to maintain biodiversity. Coastal offshore and onshore habitats have great significance on the survival of the coastal flora and fauna. Further, diversity of fungi is related to the particular habitats, abandonment of different vegetation systems and both environment and edaphic factors [6, 10, 12, 13, 15, 33]. A comparative study on composition of soil status at two sites revealed that higher concentration of fungal numbers in general and Aspergilli in particular concurred with higher moisture, low temperature and higher nutrient level at plantation site than the barren sand dunes (Table 1). All the two sites showed higher fungal numbers in rainy season followed by winter and lastly summer. But *Aspergillus* species did not exhibit seasonality. They appeared throughout the period of observation. Micro-fungi of both soils showed a positive correlation with soil moisture and total organic carbon but were negatively correlated with soil temperature. The qualitative and quantitative differences of genera and species at the two sites indicated that surface vegetation as well as nutrient composition influenced micro- fungal inhabitants of the soil [14, 31]. Similar results have been obtained from the soils at lower depth in all sampling sites. The higher population associated with plantation site may be ascribed to the greater surface area available for microbial colonization. Fungal number of two sites differed significantly (t test 5.34< p 0.01). Analysis of variance clearly indicated significant seasonal difference between the samples of soil (Table 2).

The surface mycoflora was richer in comparison to sub-surface mycoflora. Moreover, the similarity in species composition between the surface layers was found to be more akin than the subsurface soil. The species composition in soil showed marked differences with a change in habitat and surface vegetation (Table 3). The majority was from the genus *Aspergillus*; the next two in order of dominance were *Penicillium* and *Trichoderma* . Earlier reports have indicated that these genera appeared abundantly in soils [15, 34]. This may be due to the faster growth rate of these fungi in addition to their better intrinsic prolific sporulating capacity to utilize the substrate. Considering the dominant species it is clear that fungal succession in plantation site greatly differed from without plantation. A total of 177 species of fungi belonging to 71 genera were enumerated (Table 4). Species of Deutoromycotina were maximum followed by Zygomycotina and Ascomycotina. Their occurrence might be due to ability of the concerned group of fungi for survival in adverse condition and adjustment with the environment. Fifty two fungal species were detected common to site without vegetation or with *Anacardium* plantation. Out of 22 *Aspergillus* species (Table 5) isolated from two sites, soil with plantation harboured the lowest (17) while barren soil the highest one (20). *A. awamori* was recorded maximum times while contributing highest towards total population followed by *A. niger* , *A. fumigatus* ,*A. flavus* and *A. terreus* with little alterations in all the sites as reported from different parts of India [7,10,12,29,35]. Primary colonists show more rapid germination of spores, more growth and better ability to grow at lower relative humidity than secondary colonists[36] (Webster and Dix 1960). *Aspergillus* being a primary colonizer is phyllospheric in nature had important role in leaf litter decomposition [10, 15, 37, 38] . It is one of the important genus of fungi in Indian soils, dominating both in the frequency and in relative density[13,39].Restricted appearance of *A. repens, A. sydowi* at site with plantation and *A. caepitosus, A.oryzae and A.restrictus* in barren sand dunes was also observed. This is possibly due to the effect of surface vegetation of the sites corroborating Panda et al. (2009) [10].

Of the 22 species isolated 08 were common to all the sites while a few were restricted in their distribution. Interestingly, it was observed that the percentage contribution of Aspergilli in site without vegetation was more than the site with *Anacardium* plantation (Table 4). This can be attributed to the wider

ecological spectrum of the genus and low competition with other category of fungi which are less abundant in barren sand dunes compared to monoculture plantation of *A. occidentale*. The number of Aspergilli as reported here is less in comparison to its large varieties[12,40]. A large number of permutations and combinations of media and technique should be tried to unravel the innumerable Aspergilli still unreported in coastal soils of Odisha. The diversity values (H and R) show an increase as we move from sub-surface soil with plantation to surface soil without plantation (Table 6).The evenness values however show a different trend. It is highest in soils of without plantation and lowest in soil with plantation. The evenness index indicates that species were fairly evenly distributed. The surface soil of barren sand dunes have the highest species richness where as sub-surface soil of barren sand dunes shows lowest richness. The β diversity (Jacquard's index) value indicates change in species composition from one location to another (Table 7). It is similar to the findings of Wilson and Shimda (1984) [41] and Aparajita (2007) [42].It has also been observed that a change in the habitat variable can effect species composition and diversity. Thus vegetation is a major factor controlling the distribution and diversity of fungal species and subsequently deforestation can lead to loss of biological diversity as a whole.

CONCLUSION

The present study indicates that the plantation in coastal sand dunes serves as a connecting link between the distribution and diversity of fungi. However this manmade forest is not being protected stringently and facing anthropogenic disturbances. In this context, it is worthy to mention that coastal vegetation and more particularly *Annacardium occidentale* present in coastal sand dunes and their ecological role in acting as coastal shelter belts and also ensure maintenance of the biological stability as well as a healthy nutritional status of the concerned soil.

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Table 1. Soil characteristics and total fungal numbers (cfug $^{-1}$dry soil) at study sites (Average of 2 yrs data)

Sites		Temp (°c)	Moisture content (%)	pH	Total organic carbon (%)	Total nitrogen (%)	C/N ratio	P (mg/100g)	K (mg/100g)	Fungal No.x10^{-4}	*Aspergillus* No. x10^{-4}
Site without vegetation	Surface soil	34.1	.699	6.2	0.21	0.016	13.8	0.45	1.53	54	18
	Sub-surface soil	32.3	1.56	6.1	0.131	0.01	12.7	0.74	0.99	40	15
Site with *Anacardium* plantation	Surface soil	30.28	1.26	6.9	0.403	0.0246	17.2	0.2	1.7	66	20
	Sub-surface soil	28.74	2.03	6.3	0.275	0.0195	14.9	0.31	1.1	45	16

Table 2. Analysis of variance showing interactive effect of seasons and sites on fungal population of soil.

Sources	DF	SS	MSS	F value		p Value	
Varieties	3n	1164	388	7.8	4.8 *	9.8 **	23.7***
Season	2n	614	307	6.2	5.1*	10.9**	27.0***
Error	6n	298	49.7				
Total	11						

*p<0.05, ** p<0.01, *** p<0.001

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Table 3. Percentage contribution and ranks of some dominant fungi isolated from samples at study sites

	Soil from site without vegetation						Soil from site with *Anacardium* plantation					
	Surface			Subsurface			Surface			Subsurface		
Fungi	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank	No. of colony	%	Rank
Absidia butleri	14	1.99	21	15	2.27	19	45	5.32	4	38	5.12	4
A. glauca	-	-		-	-	-	23	2.72	10	19	2.56	15
A. spinosa	-	-		-	-	-	17	2.01	20	22	2.96	10
Alternaria alternate	13	1.85	22	-	-	-	-	-		-	-	
Aspergillus awamori	56	7.98	1	47	7.11	1	57	6.74	1	48	6.47	1
A. flavus	24	3.2	8	21	3.18	13	24	2.84	9	18	2.43	16
A. fonsecaceus	-	-	-	-	-	-	21	2.48	11	-	-	-
A. fumigates	28	3.99	6	27	4.08	7	25	2.96	8	21	2.83	12
A. luchuensis	18	2.56	14	21	3.17	14	18	2.13	16	13	1.75	24
A. niger	43	6.12	2	42	6.35	2	49	5.79	3	46	6.2	2
A. terreus	19	2.71	13	25	3.78	8	16	1.89	21	16	2.16	18
Chaetomium homopilatum	22	3.13	10	24	3.63	9	14	1.66	25	14	1.89	22
C.murorum	-	-	-	14	2.12	20	13	1.54	27	-	-	-
Cladosporium cladosporoides	18	2.56	15	28	4.24	5	20	2.36	12	26	3.5	9
C. oxysporum	15	2.14	19	18	2.72	16	16	1.89	22	30	4.0	8
Curvularia eragrostidis	27	3.85	7	23	3.48	10	-	-	-	16	2.16	19
C. lunata	17	2.42	16	12	1.82	21	15	1.77	23	15	2.02	20
C.pallescens	12	1.71	23	18	2.72	17	-	-	-	14	1.89	23
Drechslera australiensis	16	2.28	17	13	1.97	23	-	-	-	12	1.62	26
Fusarium species	16	2.28	18	17	2.57	18	20	2.36	13	18	2.43	17
Mucor species	-	-	-	-	-	-	13	1.66	26	-	-	-
Penicillium citrinum	30	4.27	4	28	4.24	6	44	5.2	5	33	4.45	5
P.cyaneum	-	-	-	-	-	-	-	-	-	15	2.02	21
P.javanicum	39	5.56	3	39	5.9	3	32	3.78	7	32	4.3	6
P.minio-leuteum	22	3.13	11	23	3.48	11	19	2.25	15	21	2.8	13
P. nigricans	11	1.57	24	12	1.82	22	18	2.13	17	13	1.75	25
P. oxalicum	20	2.85	12	19	2.87	15	15	1.77	24	22	2.96	11
P. rubrum	15	2.14	20	11	1.66	24	18	2.13	18	20	2.7	14
P.rugulosum	-	-	-	-	-	-	19	2.25	14	-	-	-
P. verruculosum	30	4.27	5	29	4.39	4	52	6.15	2	44	5.93	3
Rhizopus nigricans	-	-	-	-	-	-	18	2.13	19	12	1.62	27
Trichoderma viride	23	3.28	9	22	3.33	12	44	5.2	6	32	4.3	7

Table 4. Total count of fungi isolated during the study period

Sites	Total number of isolates	Total genera	Total species	*Aspergillus* species	Contribution (%)
Site without vegetation					
Surface soil	702	51	112	16	29.35
Sub Surface soil	661	37	87	11	28.13
Site with *Anacardium* plantation					
Surface soil	846	45	114	13	25.66
Sub Surface soil	742	41	93	14	23.05
Total	2951	71	177	22	26.55

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Table 5. *Aspergillus* species isolated from different sites

	Soil with plantation of *Anacardium*		Soil without plantation	
Aspergillus Species	Surface	Subsurface	Surface	Subsurface
Aspergillus awamori	+	+	+	+
A.caepitosus	-	-	+	-
A.candidus	+	+	+	+
A.carbonarius	+	+	+	+
A.flavipes	+	-	+	-
A.flavus	+	+	+	+
A.foncecaceous	+	+	+	-
A.fumigatus	+	+	+	+
A.funiculosus	+	+	+	-
A.humicola	-	+	-	-
A.luchuensis	+	+	+	+
A.niger	+	+	+	+
A.niveus	-	+	+	-
A.oryzae	-	-	-	+
A.restrictum	-	-	-	+
A.repens	-	+	-	-
A.rugulosum	-	-	+	-
A.sulphureus	-	+	-	+
A.sydowi	+	-	-	-
A.terrestre	-	-	+	-
A.terreus	+	+	+	+
A.terricola	+	-	+	-

Table 6. Dominance, diversity, evenness and richness indices of fungi in different samples at study sites

Sites	Samples	D	1-D	H	E	R
Site without vegetation	Surface soil	0.0144	0.9856	0.917	0.399	2.83
	Sub Surface soil	0.0148	0.9852	0.882	0.452	1.89
Site with *Anacardium* plantation	Surface soil	0.0112	0.9888	0.884	0.384	2.52
	Sub Surface soil	0.0102	0.9898	0.764	0.332	2.83

D= Simpson dominance index, H= Shannon diversity index, E= Evenness, R= Richness

Table 7. Comparison of different samples by coefficients of comparison

Samples	A_1	A_2	B_1	B_2
A_1	1.0	0.59	0.71	0.5
A_2		1.0	0.58	0.56
B_1			1.0	0.42
B_2				1.0

A_1=Surface soil with plantation A_2=Sub- surface soil with plantation
B_1= Surface soil without plantation B_2=Sub- surface soil without plantation